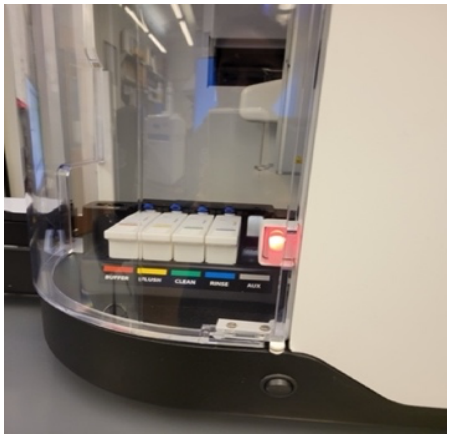
iQue Standard Operating Procedure

Things to consider while preparing sample:

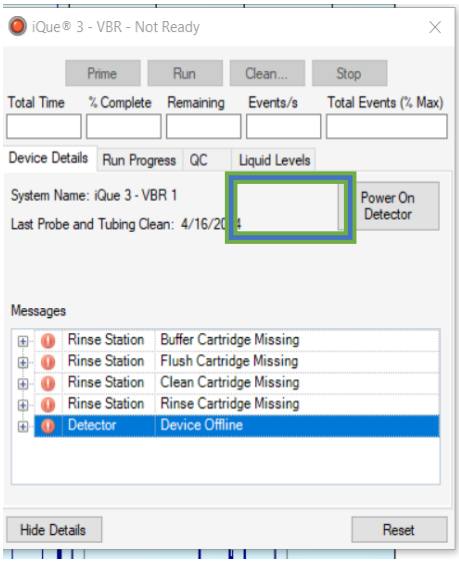
1. Ensure that there are no cell clumps, and the maximum concentration is 900 cells/uL.
2. Sartorius recommends suspending your cells/sample in QSol buffer.
3. During acquisition for every 1 second approximately 1.5 to 2uL of sample is taken up.
4. There is a 1.5-minute delay between aspiration of sample and it passing through the instrument.
5. If you are not seeing any events or seeing fewer events after 2 to 3 minutes of running in the Events/sec box, there could be clog and the cleaning and clearing the clog can take up to 60 minutes.
6. Please ensure samples are prepared properly to avoid clogs
7. The Forecyt software does not allow you to record specific number of events. It will record the number of events in a certain volume. So, the math has to be done to calculate the volume to be recorded for the number of even`ts you want to record for the concentration of cells.

Setting up an experiment:

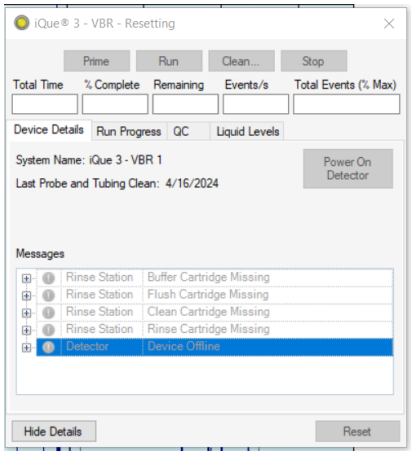
1. Red light is on when the instrument is off.



2. Check the Sheath and waste levels at the side of the instrument. Empty waste if full. If sheath is empty, please fill the bottle from iQue buffer carboy next to the instrument.
3. Please place all the cartridges in their respective positions (QSol (found in the refrigerator), Flush, Clean, Rinse (found right next to instrument))
4. To turn on the instrument start the software and click on the Power on detector.

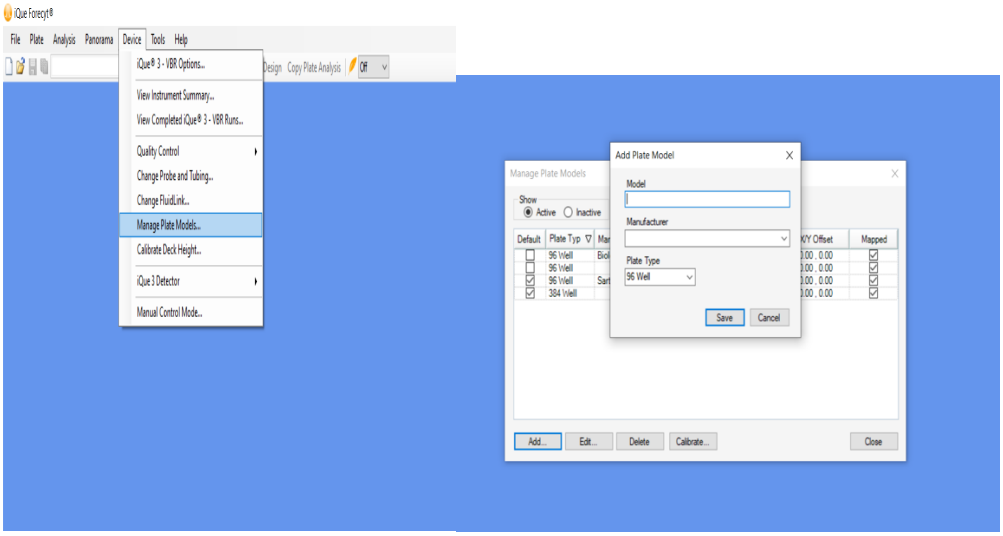


5. It takes approximately 45 minutes to start up the instrument and QC it. Core staff will start the instrument when they see it was signed up. If you are running after hours/weekends, please turn on the detector after checking the sheath and waste levels. Both the light and instrument will go into yellow and then turn green once the fluidics startup is done.

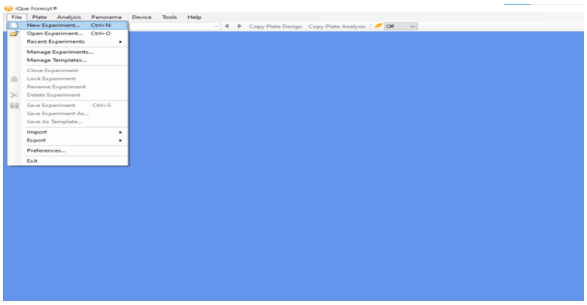


6. To check if the plate model is available to run, click on Device→ manage plate models and add the plate model and manufacturer.

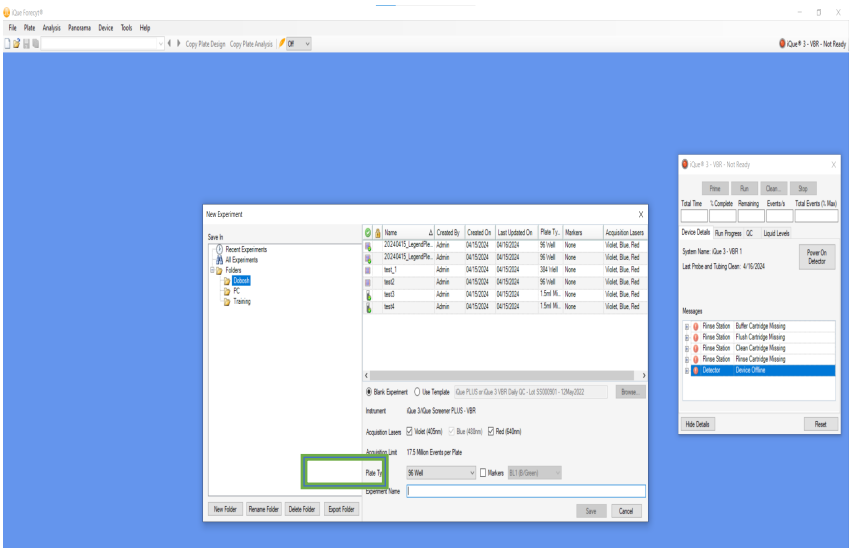
   Troubleshoot: If a particular plate is not mapped, please check if it is seated well and then select the plate and calibrate it.



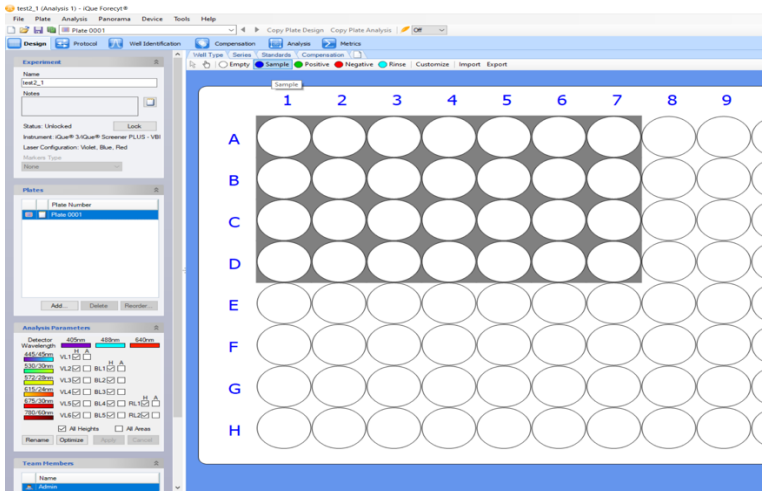
7. To make a new experiment, click on file -> new experiment. Create a new folder with your name and you can store all your experiments in that folder. Give an experiment name. Please note only 17.5 million events/plate can be recorded if you are using all three (V, B, R) lasers and 28 million events/plate if using 2 lasers and 35 million events/plate can be recorded if you are using one laser only

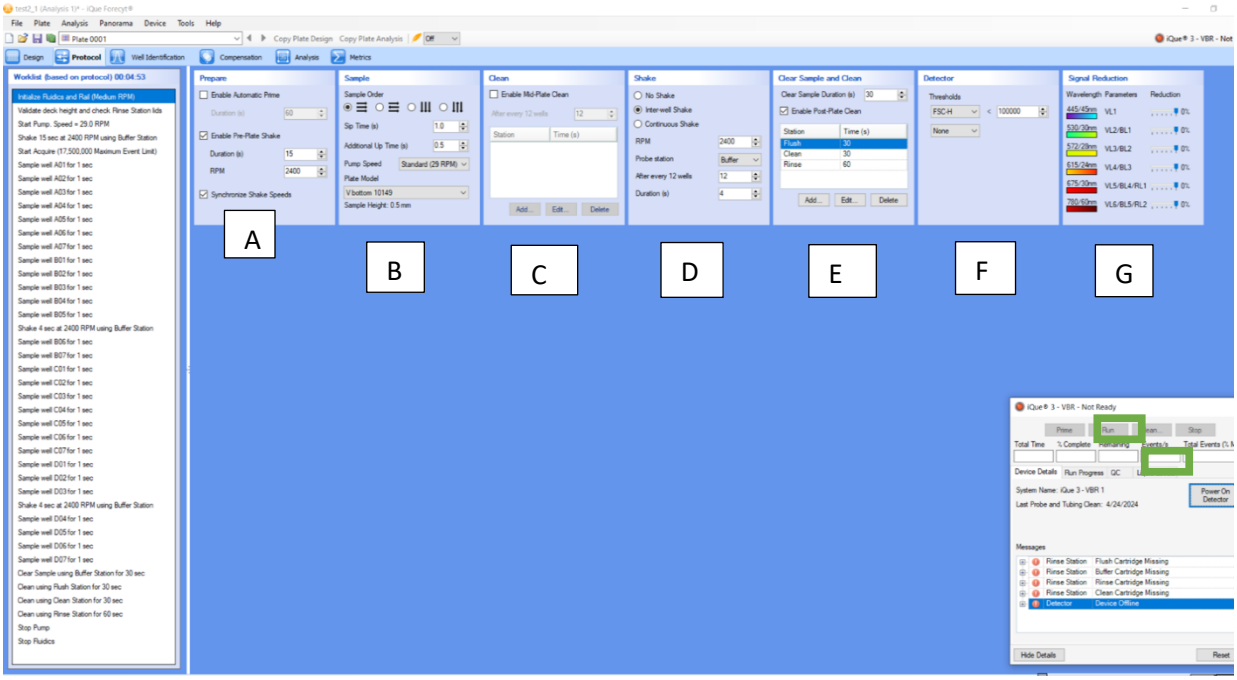


8. Choose the type of plate 96 well or 384 well and give an experiment name.



9. In your Experiment, click on design and choose all the wells that have samples. Please note that compensation on this software is done post acquisition. So, check all the well that have compensation and experimental sample and set them as sample.



10. Click on protocol and follow the steps
11. 

    A. Pre-Plate shake should be less than 500 RPM to avoid spills.
    B. Select the order to run the plate and change the Sip time based on the number of events to record.
    C. Check the enable mid plate clean to avoid clogs after every 6 to 12 wells.
    D. Inter plate shake should be again less than 500 RPM and optional
    E. Enable post plate clean if you are running multiple plates. This cleaning can be customized.
    F. Threshold can be changed.
    G. We recommend to run your first sample alone to check the signal and use signal reduction reduce the intensity of your fluorochrome/s.

12. After designing your protocol, click run. Each plate takes 20 to 30 minutes to complete running. Check for events/sec. If it is showing no events or less number of events, then there is a possible clog. Stop running and do a clean cycle.
13. After the samples are run, analysis can be done using the Forecyt software or transfer the FCS files to analyze in Flowjo.

Standard Operating Procedure			
iQUE 3 SOP	**Approval Date**	**Approved By**	
	10/29/2024		
Revision History			
Revision Date	**Version #**	**Change**	**Sections**
10/29/24	1.00	New	N/A